Journal of Assisted Reproduction and Genetics, Vol. 20, No. 5, May 2003 (© 2003)

Andrology

High Sex Chromosome Aneuploidy and Diploidy Rate of Epididymal Spermatozoa in Obstructive Azoospermic Men

Nares Sukcharoen,[1,3] Jiraporn Ngeamvijawat,[1] Tippawan Sithipravej,[2] and Sakchai Promviengchai[2]

Submitted May 13, 2002; accepted February 17, 2003

***Purpose*:** To evaluate the frequencies of sex chromosome aneuploidy and diploidy rate of epididymal spermatozoa from obstructive azoospermic men and its impact on intracytoplasmic sperm injection (ICSI) outcomes.
***Methods*:** Epididymal spermatozoa retrieved from 24 obstructive azoospermic men and ejaculated spermatozoa from 24 fertile donors were analyzed using triple color fluorescence in situ hybridization (FISH) techniques, in order to investigate the rates of diploidy and aneuploidy for chromosomes 18, X and Y.
***Results*:** Epididymal spermatozoa from obstructive azoospermic men had total sex aneuploidy, disomy 18, and diploidy rates significantly higher than ejaculated spermatozoa from normozoospermic fertile controls (1.44% vs. 0.14%, 0.11% vs. 0.02%, and 0.18% vs. 0.02%, respectively; $p < 0.005$). There were no statistically significant differences in ICSI outcomes between the patients who had high and low epididymal sperm aneuploidy rate.
***Conclusions*:** Epididymal spermatozoa from obstructive azoospermic patients had an elevated sex chromosome aneuploidy and diploidy rate. The increased frequency of chromosomal abnormalities did not have a direct effect on the ICSI outcome.

KEY WORDS: Aneuploidy; diploidy; epididymal sperms; FISH; ICSI; PESA; sex chromosomes.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) has developed for the treatment of severe male factor infertility. It allows the use of spermatozoa from men with severely compromised semen parameters and currently is used even in some cases of azoospermia where spermatozoa can be retrieved from the epididymis or testis. Although ICSI has demonstrated its safety in many large programs worldwide, its use for the treatment of male infertility has some negative impacts on the genetic composition of the human population for future generations (1), especially about the possible high risk of chromosomal aneuploidies from paternal origin in the children (2). This concern has been confirmed by several reports of a higher incidence of sex chromosomal aneuploidies of paternal origin in children conceived after ICSI, compared to the general population (3–5).

Many reports have demonstrated that men with various forms of infertility have an increased risk of chromosomal abnormalities in their ejaculated spermatozoa (6–9) but very few studies have reported the risk of chromosomal abnormalities in epididymal spermatozoa from obstructive azoospermic men (10,11). The fluorescence in situ hybridization (FISH) technique provides a rapid and reliable source of data, allowing for testing of the most frequently involved chromosomes in large numbers of cells in a short time. In this study, the results of sperm chromosomal analysis by three-color FISH analysis of epididymal spermatozoa from 24 obstructive azoospermic men

[1] Department of Obstetrics and Gynaecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.
[2] Nawabutra Center for Assisted Reproduction and Embryology, Bangkok, Thailand.
[3] To whom correspondence should be addressed; e-mail: sukcharoen@yahoo.com.

were reported and compared with the ejaculated spermatozoa from healthy fertile controls. The ICSI outcome using epididymal spermatozoa in terms of fertilization rate, implantation rate, and pregnancy rate were also analyzed to investigate the potential effect of the chromosomal abnormalities on the ICSI outcome.

METHODS

Obstructive Azoospermic Patients

Twenty-four obstructive azoospermic men were recruited in this study. All of the patients were treated by the ICSI using the epididymal spermatozoa retrieved by percutaneous epididymal sperm aspiration (PESA). The mean age of the azoospermic men was 43.2 ± 8.7 years, the mean age of the females was 31.6 ± 4.0 years. All men had normal testicular volume, normal serum FSH concentration. We also recruited 24 healthy fertile men with normal semen analysis according to the WHO reference (12) as a control group.

Ovarian Stimulation

Briefly, ovarian stimulation was performed with two protocols (down-regulation and flare-up) using the GnRH agonist (GnRH-a) buserelin acetate in combination with recombinant FSH (13). The initial dosage of recombinant FSH was individualized based on day 3 FSH or the response from the previous stimulation. Changes in recombinant FSH dosage were based on follicular development as reflected by changes in follicular diameter and number assessed by serial transvaginal sonography. Five thousand international units of hCG (Profasi; Serono Laboratories, Randolph, MA) was administered intramuscularly when the patient had at least three follicles with a diameter of 18 mm. Transvaginal follicular aspiration was performed 36 h later.

Oocyte Preparation and Handling

The oocyte preparation and handling for complete removal of the corona cells were carried out as described previously (13). Oocytes were assessed for nuclear maturity by examination for the first polar body and the presence or absence of a germinal vesicle.

Percutaneous Sperm Aspiration (PESA)

PESA took place in the operating room using intravenous sedation anesthesia with Propofol and local anesthesia with Bupivacaine injected at the skin and underneath the epididymal caput. The epididymal caput was identified and held firmly between thumb and index finger. A small needle (26 gauge) was connected to a 1-mL disposable syringe. The proximal part of the epididymal caput was punctured. Suction was applied to the syringe and the needle was withdrawn gradually to the point where segments of fluid from the epididymis were seen entering the syringe. The aspirate was then flushed out of the needle and syringe into a sterile petri dish using HEPES-buffered Ham's F10 supplemented with 10% patient serum. This procedure can be performed as many times as necessary until sufficient sperms are recovered for ICSI (14). The remaining epididymal spermatozoa were divided for cryopreservation and preparation for FISH.

Intracytoplasmic Sperm Injection (ICSI) Procedure

ICSI was performed as described previously (13). Microinjection was preserved only for the mature oocytes that extruded their first polar bodies.

Assessment of Fertilization and Pregnancy Outcome

About 16–18 h after the microinjection, the oocytes were observed under the inverted microscope ($\times 200$ or $\times 400$ magnification) for any sign of damage that may have been caused by microinjection and for the presence of pronuclei and polar bodies. Fertilization was considered normal when two clearly distinct pronuclei containing nucleoli were present. After an additional 24–30 h of in vitro culture, embryos were examined under the microscope to assess their developmental stage and quality on the basis of their morphological aspects. Depending on the day of embryo transfer, embryo cleavage was judged 3, 4, and 5 days after the ICSI procedure. A maximum of three cleaving embryos were then transferred into the uterine cavity. Supernumerary embryos were cryopreserved for the next embryo transfers. The luteal phase was supplemented with natural progesterone pessaries (Cyclogest® 400 mg, Hoechst, Hounslow, U.K.) given daily as described previously (15).

Successful implantation was determined 14 days after embryo transfer by serum β-hCG. A clinical pregnancy was confirmed as the presence of at least one gestational sac with a fetal heartbeat by transvaginal

sonography performed 3 weeks later after detection of β-hCG. Implantation rate was defined as the ratio of the number of gestational sacs containing a fetus with heart activity and the number of transferred embryos. The clinical pregnancies reaching 20 weeks of gestation were considered ongoing. Subsequent ongoing pregnancy and delivery were also included. This clinical study was approved by the local ethics committee.

Sperm Preparation and Fixation

The sperm samples were washed three times in phosphate-buffered saline (PBS; 0.15 mol/L NaCl, 10 mmol/L sodium phosphate, pH 7.2), centrifuged at 280*g* for 10 min and the sediment was then dropped onto precleaned glass slides and air dried. At least two slides were prepared for each patient. The slides were stored at −20°C until evaluation.

Fluorescence In Situ Hybridization (FISH)

Sperm Decondensation. The sperm decondensing procedure was performed following a previously described protocol (16), the air dried slides were incubated at room temperature for 5 min in 0.01 M dithiothreitol (DTT) in 0.1 M Tris, pH 8.0, and in 0.01 M LIS (3,5-diioiosalicylic acid, lithium salt) and 0.001 M DTT in 0.1 M Tris, pH 8.0, for 10 min. Then slides were rinsed in 2× SSC, pH 7.0, and allowed to air dry.

Chromosome Specific DNA Probes. Simultaneous three-probe three-color FISH was performed using probe sets for either chromosomes X, Y, and 18. Three-probe FISH was used to differentiate disomy from diploidy. Simultaneous scoring of three chromosomes also provided an internal control to differentiate nullisomy from hybridization failure (no signals).

The DNA probes (Vysis Inc., Framingham, MA) used in this study recognize the satellite III DNA of the chromosomes Y (Yq12, locus DYZ1), alpha satellite DNA of the centromeric region of human chromosomes X (Xp11.1–q11.1, locus DXZ1), and chromosomes 18 (18p11.1–q11.1, locus D18Z1). The probes detecting chromosome X, Y, and 18 were labeled with fluorescent haptens CEP (chromosome enumeration probe) SpectrumOrange, CEP SpectrumGreen, and CEP SpectrumAqua, respectively.

In Situ Hybridization to Decondensed Sperm. The slides were dehydrated through an ethanol series (80, 95, and 100% ethanol) and air dried. The hybridization solution was prepared by mixing 7 mL of Spectrum CEP hybridization buffer, 1 mL of X SpectrumOrange, 1 mL of Y SpectrumGreen, and 0.5 mL of 18 SpectrumAqua. The mixture was vortexed thoroughly, centrifuged for 1–3 s, and left at room temperature for a short time. For the simultaneous denaturation of slides and probes, a 10 μL of hybridization mixture containing 7 μL of hybridization solution and 2 μL of purified water was applied to each slide, sealed under a coverslip with rubber cement, then denatured at 72°C for 10 min, and hybridized.

The process of FISH was carried out according to the manufacturer's recommendation (Vysis). Briefly, slides were denatured at 75°C for 5 min. Slides were air-dried, and warmed to 45–50°C before the denatured probe mixture was applied. The area with the probe mixture was covered with a coverslip, sealed with rubber cement, and the slides were placed in a humidified box in a 37°C incubator. Posthybridization washes were carried out after overnight hybridization incubation. Slides were first immersed in three changes of 50% formamide/2× SSC for 10 min each, and then washed for 5 min in 2× SSC for another 10 min. Slides were finally washed and incubated at 45°C. Finally, the slides were mounted with glycerol: PBS mixture containing 0.5 μg/mL of 4,6-diamidino-2-phenylindole (DAPI II; Vysis Inc., Framingham, MA) as a nuclear counterstain and then sealed with nail vanish and stored in the dark at −20°C.

Fluorescence Microscopy. FISH signals were analysed with an epifluorescence microscope (Olympus, BX50, Japan) equipped with an appropriate triple bandpass filter set for SpectrumOrange™, SpectrumGreen™, SpectrumAqua™, and DAPI II (Vysis). A spectrum nucleus was scored only if it was intact and not overlapped. An X or Y chromosome in a sperm nucleus was recognized by an orange or a green fluorescent spot, respectively. Chromosome 18 was recognized by the presence of an aqua fluorescent spot in the sperm nucleus. At least 2000 spermatozoa were evaluated in each sample from healthy fertile men and all epididymal sperms retrieved, left from ICSI and cryopreservation, were evaluated.

Scoring Criteria. Sperm nuclei scoring was done according to the criteria described previously (17). Nuclei were scored only if they were not overdecondensed, did not overlap, and were intact with clearly defined borders. Normal haploid sperm nuclei carried one signal for a sex chromosome and one signal for an autosome. Sperm nuclei were scored as disomic for sex chromosomes when an extra X or Y signal and a single aqua fluorescent spot were clearly visible within the nucleus and the distance between the

two fluorescent signals was equal to or greater than the diameter of one fluorescent domain. Two spots separated by less than the diameter of one domain were scored as a single signal (18). The absence of signal for a single chromosome was scored as nullisomy for that chromosome. Sperm nuclei were considered diploid when an extra X or Y chromosome signal and two chromosome 18 signals were present. Diploid cells with a clearly defined round shape and without a tail were considered spermatogenetic or other cells and were not scored. Scoring was done blindly on coded samples whose origins were unknown to the individuals involved in the scoring.

Statistical Analysis. Descriptive statistics have been performed for each variable, quantitative results were presented by using mean and standard deviations, qualitative results were summarized by using distribution of frequencies.

Before comparing the two groups, each variable was tested in order to check the normality distribution using Kolmogorov–Smirnov test, the comparisons of means was performed using a two-sample unpaired t-test or Mann–Whitney test. Proportions for the two groups were compared using a χ^2 test and a Fisher's exact test, where applicable. All statistical analysis were performed using the SPSS for Windows, version 10.05 (SPSS Inc., Chicago, IL). A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

A total of 23,267 epididymal spermatozoa from the 24 obstructive azoospermic patients and 48,000 ejaculated spermatozoa from the 24 healthy fertile controls were analyzed by three-color FISH with DNA probes for chromosomes X, Y, and 18. A mean of 969 spermatozoa (range, 100–3057 spermatozoa) and 2000 spermatozoa were analyzed per sample in study group and control group, respectively. Hybridization was efficient, with an overall frequency of hybridization of 99.51% (range, 98.77–100%) and 99.68% (range, 99.25–99.85%) in study group and control group, respectively. No difference in hybridization efficiency between the patients and controls was observed. The details of the frequencies of X- and Y-bearing sperm, sex disomy, disomy 18, and diploidy of epididymal spermatozoa from the obstructive azoospermic men are shown in Table I. No nullisomic spermatozoa were found in both groups.

The results of FISH analysis of epididymal spermatozoa from the obstructive azoospermic patients compared with the results obtained from healthy fertile controls are shown in Table II. Statistically significant differences were observed in the frequencies of haploidy X, haploidy Y, sex aneuploidy, disomy 18, and diploidy between the study group and the control group ($p < 0.005$).

The patients were divided into two groups according to the causes of obstructive azoospermia: the patients with history of vasectomy and failed vasectomy ($n = 12$) and the remaining patients with other causes of obstruction ($n = 12$). A total of 23,267 spermatozoa were scored: 13,524 in the vasectomized men and 9743 in the remaining obstructive azoospermic patients. There were no significant difference in sex disomy rate (1.37% vs. 1.54%), disomy 18 rate (0.10% vs. 0.13%), diploidy rate (0.16% vs. 0.21%) between vasectomized men and the remaining obstructive azoospermic patients, respectively. Moreover, the total aneuploidy rate did not correlate to the duration of vasectomy ($r = 0.048$, $p = 0.89$).

Comparison of the ICSI Outcome

An approach toward assessing the relationship between total sperm aneuploidy rate and ICSI outcome was to compare those cases exhibiting low and high total sperm aneuploidy rate. For this purpose, the median of the total sperm aneuploidy rate was calculated, which was equal to 1.5%. The ICSI outcomes of the cases who had total sperm aneuploidy rate less than 1.5% (Group I) were compared with those whose total sperm aneuploidy rate was more than 1.5% (Group II) (Table III).

A total of 24 obstructive azoospermic patients underwent 30 consecutive ICSI cycles. The mean female age and male age were not significantly different between both groups. There was no statistically significant difference in mean numbers of MII oocytes between the two groups. The ICSI outcome, included fertilization rate, cleavage rate, implantation rate, pregnancy rate, and delivery rate were also similar for both groups.

DISCUSSION

Aneuploidy is one of the most serious and common chromosomal abnormalities affecting human embryos and offspring. For the general population, numerical chromosomal abnormalities are mostly of maternal origin, usually resulting from nondisjunction, which is strongly related to maternal age. However, it can also be transmitted via sperm (19).

Table I. Frequencies (as Percentages) of Numerical Chromosomal Abnormalities in Obstructive Azoospermic Patiens

Obstructive azoospermic patients	Sex chromosome			Total sex aneuploidy rate (%)	Disomy 18 (%)	Diploidy rate (%)
	XX (%)	XY(%)	YY (%)			
P1	2.01	2.69	1.01	5.71	0.34	1.01
P2	0.69	1.55	0.52	2.76	0.17	0.35
P3	0.75	1.05	0.30	2.09	0.15	0.45
P4	2.02	4.04	1.01	7.07	0.00	1.01
P5	0.71	1.00	0.30	2.01	0.12	0.12
P6	0.13	0.53	0.16	0.82	0.10	0.03
P7	0.11	1.01	0.11	1.24	0.23	0.34
P8	0.34	0.79	0.00	1.13	0.00	0.00
P9	0.13	0.60	0.13	0.87	0.07	0.20
P10	0.25	0.75	0.33	1.34	0.08	0.17
P11	0.31	0.82	0.20	1.33	0.10	0.20
P12	0.37	1.10	0.27	1.74	0.00	0.00
P13	0.17	0.75	0.08	1.01	0.17	0.08
P14	0.16	0.80	0.16	1.12	0.00	0.48
P15	0.30	1.29	0.10	1.69	0.30	0.10
P16	0.28	0.66	0.47	1.41	0.19	0.09
P17	0.11	0.56	0.22	0.90	0.00	0.00
P18	0.20	0.61	0.10	0.91	0.00	0.10
P19	0.31	0.81	0.20	1.32	0.00	0.20
P20	0.31	1.07	0.31	1.68	0.15	0.61
P21	0.78	1.72	0.16	2.66	0.00	0.00
P22	0.45	0.90	0.11	1.46	0.23	0.34
P23	0.23	0.45	0.11	0.79	0.23	0.00
P24	0.76	1.51	0.50	2.77	0.25	1.01

Recently, the incidence of chromosomal abnormalities from paternal origin in children born after ICSI has been a matter of concern (3–5).

FISH with the use of DNA probes for specific chromosomes has become an increasingly popular approach for estimating aneuploidy frequencies in spermatozoa because it provides a rapid and reliable result. Using multicolor FISH techniques, a higher frequency of chromosomal aneuploidies in spermatozoa of infertile men compared to normal fertile donors has been reported (6–9,20). In this study, we used three-color FISH with centromeric DNA probes for chromosomes X, Y, and 18. DNA probes for chromosome 18 was used as an internal autosomal control. It allowed the differentiation between disomic and diploid spermatozoa and also between failure of hybridization and nullisomic spermatozoa.

Table II. Results of Fluorescence In Situ Hybridization Analysis in 24 Obstructive Azoospermic Patients Compared With Healthy Fertile Controls

Findings	No. (%) in control group	No. (%) in study group	*p* value
Haploid X	23,975 (49.95)	11,314 (48.63)	<0.001
Haploid Y	23,763 (49.51)	11,311 (48.61)	<0.005
Sex aneuploidy	69 (0.14)	335 (1.44)	<0.001
Disomy 18	8 (0.02)	26 (0.11)	<0.001
Diploidy	11 (0.02)	42 (0.18)	<0.001
Total	48,000	23,267	

In this study, we analyzed the rates of aneuploidy for chromosomes 18, X, and Y in 24 obstructive azoospermic men and in 24 fertile donors using triple-color FISH techniques. The overall hybridization efficiency was about 99.6% that was similar to the previous study (99.8%) (21). The hybridization efficiency were high in both patients and controls (99.51% vs. 99.68%, respectively), which were not statistically different.

The reported frequencies of chromosomal abnormalities of ejaculated spermatozoa from healthy fertile men range from 0.05 to 0.45% for disomy 18 (7,9,20–22), 0.23–0.78% for overall sex chromosome aneuploidy (7,9,10,20,21,23), 0–0.17% for XX disomy, 0–0.62% for YY disomy, and 0–0.3% for XY disomy (20,22,23); the diploidy rates range from 0 to 0.25% (7,9,10,20–22). The frequencies of sex chromosome aneuploidy (0.14%), disomy 18 (0.02%), and diploidy (0.02%) observed in our control group were lower than the previous studies (7,9,10,20–22) because the healthy fertile men were recruited as controls and all of them had normal semen analyses according to WHO criteria. The variability of these frequencies may be from the various geographical areas, criteria

Table III. Characteristics of the Patients and the ICSI Outcomes

	Total sperm aneuploidy rate		
	Group I (<1.5%)	Group II (>1.5%)	
Characteristics of the patients			
No. of couples	12	12	
Group I	6	6	
Group II	6	6	
No. of cycles	18	12	
Age (years)			
Male	42.1 ± 8.1	44.4 ± 9.7	NS
Female	31.8 ± 3.1	32.3 ± 2.6	NS
Outcome of PESA and ICSI			
Total no. of retrieved oocytes	223	143	
Total no. of MII oocytes	162	112	
Mean no. of MII oocytes	8.9 ± 5.1	9.5 ± 5.7	NS
Overall fertilization rate	127/162 (78.4%)	83/112 (74.1%)	
Fertilization rate	76.1 ± 15.9	75.5 ± 17.1	NS
Cleavage rate	95.2 ± 9.0	96.2 ± 6.0	NS
No. of transfers	27	13	
No. of embryos transferred	82	37	
No. of positive hCG	10	9	
Implantation rate	10/162 (6.2%)	9/112 (8.0%)	NS
No. of biochemical pregnancies	1	0	
No. of anembryonic pregnancy	1	0	
No. of ectopic pregnancies	3	0	
No. of deliveries	5/12 (41.7%)	4/11 (36.4%)	NS
Singleton	5	2	
Twins	0	2	

Note. Implantation rate = number of implanted embryos per number of embryos replaced in the uterine cavity; PR per transfer = number of pregnancy per number of embryo transfers.

for fertile men selection, the different FISH protocols: decondensation procedures, types of probes, and the different scoring criteria.

The reported frequencies of chromosomal abnormalities of epididymal spermatozoa from obstructive azoospermic men range from 2.89 to 4.36% for overall sex chromosome aneuploidy, 0.54% for disomy 18, and 0.44% for diploidy (10,11). However, very small numbers of obstructive azoospermic men were recruited and very small numbers of epididymal spermatozoa per case were analyzed in these studies. The frequencies of sex chromosome aneuploidy (1.44%), disomy 18 (0.11%), and diploidy (0.18%) observed in our study group were lower than the previous studies (10,11). The difference in the frequencies of aneuploidies may be due to the limited numbers of spermatozoa analyzed in the previous studies, the different etiologies of obstructive azoospermic men, or the differences in FISH methodology. The proportions of aneuploidy involving the sex chromosomes in our study were significantly higher than the rate for chromosome 18, which was similar to the previous studies (9,10). These results support the possibility of a paternal origin of sex chromosome abnormalities in the karyotype of ICSI offspring.

We also compared the aneuploidy rates of the sex chromosomes and chromosome 18 between the patients and controls. A higher frequency of the chromosome aneuploidy rate of the epididymal spermatozoa from obstructive azoospermic patients was observed as compared to the ejaculated sperm from normal fertile controls ($p < 0.001$) (Table II). The aneuploidy and diploidy rate of epididymal spermatozoa in obstructive azoospermic men in our study are about 10 times higher than ejaculated spermatozoa in normal fertile men.

The frequencies of chromosomal abnormalities of epididymal spermatozoa in our study were significantly increased in the obstructive azoospermia men, which is similar to the previous studies (10,24). In previous study, epididymal spermatozoa from obstructive azoospermic men had sex chromosome aneuploidy, total aneuploidy, and diploidy rates significantly higher than ejaculated spermatozoa from normozoospermic controls (2.89% vs. 0.81%, 4.84% vs. 1.47%, and 0.44% vs. 0.13%, respectively). Sex chromosome aneuploidy rate was 2.89%. However, only six obstructive azoospermic men were studied and five of six men were CABVD (10). In other study, epididymal spermatozoa from 10 obstructive

azoospermic men were reported to have total aneuploidy rate significantly higher than ejaculated spermatozoa from normozoospermic controls (8.2% vs. 1.6%) (11). In recent study, epididymal spermatozoa from eight obstructive azoospermic men were reported to have total aneuploidy rate similar to ejaculated spermatozoa from normozoospermic controls (1.8% vs. 1.5%). All of the patients were CABVD. However, very small numbers of epididymal spermatozoa were analyzed in this study (25).

The differences in chromosomal abnormalities between ejaculated sperm and epididymal spermatozoa in obstructive azoospermic men may be explained by the sequestration of the abnormal epididymal spermatozoa during maturation and passage of sperm in the epididymis (11) and abnormal spermatogenesis from high back pressure in the seminiferous tubules in the obstructive azoospermic men.

The patients were divided into two groups according to their causes of obstructive azoospermia, 12 were obstructive azoospermic men from vasectomy with failed vasectomy reversal and 12 were obstructive azoospermic men from other causes. There were no significant differences in sex chromosome aneuploidy, disomy 18, and diploidy rates between these two groups. Surprisingly, the frequencies of chromosomal abnormalities of epididymal spermatozoa were also significantly increased in obstructive azoospermic men, even in the previous fertile men after vasectomy and failed vasectomy reversal. Although vasectomized men were previously fertile men, the rate of total sex chromosome aneuploidy, disomy 18, and diploidy were significantly higher than fertile healthy controls. However, relationship between the duration of vasectomy and the frequency of chromosome abnormalities cannot be demonstrated. Overall findings suggest the need for future research to study whether long duration of vasectomy may increase the frequency of sperm aneuploidies.

Since aneuploidy might have a negative impact on oocyte fertilization and/or on embryonic development, the rate of epididymal sperm aneuploidy in obstructive azoospermic patient undergoing ICSI and its impact on ICSI results were evaluated by comparing the ICSI outcome between the obstructive azoospermic patients who had the total aneuploidy rate higher and lower than the median value. There were no statistically significant differences of ICSI outcomes between both groups.

In agreement with the previous study about the relation of incidence of ejaculated sperm aneuploidy and assisted reproductive outcome (26), the increased frequency of chromosomal abnormalities of epididymal spermatozoa did not have a direct effect on the ICSI outcome, including fertilization rate, pregnancy rate, and pregnancy outcome. However, some previous studies found the association between the adverse ICSI outcomes and an increased aneuploidy rate of ejaculated spermatozoa (8,9). This is probably due to the small sample size and the frequencies of epididymal spermatozoa aneuploidy are not high enough to affect the overall ICSI outcomes. In conclusion, the increased frequency of chromosomal abnormalities did not have a direct effect on the fertilization rate, pregnancy characteristics, or outcomes in our study. Although the clinical outcomes of ICSI using epididymal spermatozoa from the groups that had different sperm aneuploidy rates were similar, the long-term follow-up of the ICSI outcome using epididymal spermatozoa in larger population is needed.

In summary, our results show increased rates of diploidy and disomy in epididymal spermatozoa from obstructive azoospermic men compared with ejaculated spermatozoa from healthy fertile controls. Moreover, there were no differences in the incidence of epididymal spermatozoa chromosomal aneuploidy between the vasectomized men and men with other causes of obstructive azoospermia. Where epididymal spermatozoa were used for ICSI in obstructive azoospermia, the differences of percentage of chromosome abnormalities did not influence ICSI outcome. Pregnancy occurred even in the presence of an elevated sperm aneuploidy rate, thus increasing the risk of generating offspring with chromosomal abnormalities in this group. This study can provide the data for counseling the patients about the potential paternal contribution to the risk of fetal chromosomal anomalies after ICSI in obstructive azoospermic men.

ACKNOWLEDGMENT

This study was supported by the Thai National Budget for Research and Development.

REFERENCES

1. Johnson MD: Genetic risks of intracytoplasmic sperm injection in the treatment of male infertility: Recommendations for genetic counseling and screening. Fertil Steril 1998;70:397–411
2. Van Opstal D, Los FJ, Ramlakhan S, Van Hemel JO, Van Den Ouweland AM, Brandenburg H, Pieters MH, Verhoeff A, Vermeer MC, Dhont M, In't Veld PA: Determination of the parent origin in nine cases of prenatally detected chromosome

aberrations found after intracytoplasmic sperm injection. Hum Reprod 1997;12:682–686
3. Bonduelle M, Camus M, De Vos A, Staessen C, Tournaye H, Van Assche E, Verheyen G, Devroey P, Liebaers I, Van Steirteghem A: Seven years of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. Hum Reprod 1999;14:243–264
4. Loft A, Petersen K, Erb K, Mikkelsen AL, Grinsted J, Hald F, Hindkjaer J, Nielsen KM, Lundstrom P, Gabrielsen A, Lenz S, Hornnes P, Zisbe S, Ejdrup HB, Lindhard A, Zhou Y, Nyboe Andersen A: A Danish national cohort of 730 infants born after intracytoplasmic sperm injection (ICSI) 1994–1997. Hum Reprod 1999;14:2143–2148
5. Aboulghar H, Aboulghar M, Mansour R, Serour G, Amin Y, Al-Inany H: A prospective controlled study of karyotyping for 430 consecutive babies conceived through intracytoplasmic sperm injection. Fertil Steril 2001;76:249–253
6. Pang MG, Hoegerman SF, Cuticchia AJ, Moon SY, Doncel GF, Acosta AA, Kearns WG: Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in-situ hybridization in spermatozoa from nine patients with oligoastheno-teratozoospermia undergoing intracytoplasmic sperm injection. Hum Reprod 1999;14:1266–1273
7. Ushijima1 C, Kumasako Y, Kihaile PE, Hirotsuru K, Utsunomiya T: Analysis of chromosomal abnormalities in human spermatozoa using multi-colour fluorescence in-situ hybridization. Hum Reprod 2000;15:1107–1111
8. Van Dyk Q, Lanzendorf S, Kolm P, Hodgen GD, Mahony MC: Incidence of aneuploid spermatozoa from subfertile men: Selected with motility versus hemizona-bound. Hum Reprod 2000;15:1529–1536
9. Rubio C, Gil-Salom M, Simon C, Vidal F, Rodrigo L, Minguez Y, Remohi J, Pellicer A: Incidence of sperm chromosomal abnormalities in a risk population: Relationship with sperm quality and ICSI outcome. Hum Reprod 2001;16:2084–2092
10. Bernardini L, Gianaroli L, Fortini D, *et al.*: Frequency of hyper-, hypohaploidy and diploidy in ejaculate, epididymal and testicular germ cells of infertile patients. Hum Reprod 2000;15:2165–2172
11. Levron J, Aviram-Goldring A, Madgar I, Raviv G, Barkai G, Dor J: Sperm chromosome abnormalities in men with severe male factor infertility who are undergoing in vitro fertilization with intracytoplasmic sperm injection. Fertil Steril 2001;76:479–484
12. World Health Organization: WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction, 4th edn. Cambridge, England, Cambridge University Press, 1999
13. Sukcharoen N, Sithipravej T, Promviengchai S, Boonkasemsanti W: Sperm morphology evaluated by computer (IVOS) in prediction of fertilization rate in vitro after intracytoplasmic sperm injection (ICSI). Fertil Steril 1998;69:564–568
14. Sukcharoen N, Sithipravej T, Promviengchai S, Boonkasemsanti W: Comparison of the fertilization rate after intracytoplasmic sperm injection (ICSI) using ejaculated sperm, epididymal sperms, and testicular sperms. J Med Assoc Thai 1998;81:565–571
15. Sukcharoen N, Sithipravej T, Promviengchai S, Boonkasemsanti W: No differences in outcome of surgical sperm retrieval with ICSI in different post-vasectomy intervals. Fertil Steril 2000;74:174–175
16. Wyrobek AJ, Alhborn T, Balhorn R, Stanker L, Pinkel D: Fluorescence in situ hybridization to Y chromosomes in decondensed human sperm nuclei. Mol Reprod Dev 1990;34:308–313
17. Blanco J, Egozcue J, Vidal F: Incidence of chromosome 21 disomy in human spermatozoa as determined by fluorescent in-situ hybridization. Hum Reprod 1996;11:722–726
18. Martin RH, Rademaker A: Reliability of aneuploidy estimates in human sperm: Result of fluorescence *in situ* hybridization studies using two different scoring criteria. Mol Reprod Dev 1995;42:89–93
19. Wyrobek AJ: Methods and concepts in detecting abnormal reproductive outcomes of paternal origin. J Reprod Toxicol 1993;7:3–16
20. Vegetti W, Van Assche E, Frias A, Verheyen G, Bianchi MM, Bonduelle M, Liebaers I, Van Steirteghem A: Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence *in-situ* hybridization in infertile men. Hum Reprod 2000;15:351–365
21. Aran B, Blanco J, Vidal F, Vendrell JM, Egozcue S, Barri PN, Egozcue J, Veiga A: Screening for abnormalities of chromosomes X, Y, and 18 and for diploidy in spermatozoa from infertile men participating in an in vitro fertilization-intracytoplasmic sperm injection program. Fertil Steril 1999;72:699–701
22. Calogero AE, De Palma A, Grazioso C, Barone N, Romeo R, Rappazzo G, D'Agata R: Aneuploidy rate in spermatozoa of selected men with abnormal semen parameters. Hum Reprod 2001;16:1172–1179
23. Martin RH, Greene C, Rademaker A, Barclay L, Ko E, Chernos J: Chromosome analysis of spermatozoa extracted from testes of men with non-obstructive azoospermia. Hum Reprod 2000;15:1121–1124
24. Viville S, Warter S, Meyer JM, Wittnmer C, Lriot M, Mollard R, Jacqmin D: Histological and genetic analysis and risk assessment for chromosomal aberration after ICSI for patients with CABVD. Hum Reprod 2000;15:1613–1618
25. Palermo GD, Colombero LT, Hariprashad J, Schlegel PN, Rosenwaks Z: Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI. Hum Reprod 2002;17:570–575
26. Colombero LT, Hariprashad JJ, Tsai MC, Rosenwaks Z, Palermo GD: Incidence of sperm aneuploidy in relation to semen characteristics and assisted reproductive outcome. Fertil Steril 1999;72:90–96